AD/A-003 073

HYPERBARIC-HYPOBARIC INTERACTIONS AS THEY RELATE TO COMPRESSED AIR DIVING AND AVIATION: CANINE EXPERIMENT

James L. Kupper, et al

Naval Aerospace Medical Research Laboratory
Pensacola, Florida

November 1974

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D AD/A-003073

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Naval Aerospace Medical Research Laboratory
Pensacola, Florida 32512

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
N/A

3. REPORT TITLE
HYPERBARIC-HYPOBARIC INTERACTIONS AS THEY RELATE TO COMPRESSED AIR DIVING AND AVIATION: CANINE EXPERIMENT

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
N/A

5. AUTHOR(S) *(First name, middle initial, last name)*
James L. Kupper, Lt Colonel, USAF VC; Walter P. Trevethan, CPT, VC USAR; and Richard J. Brown, Lt Colonel, USAF VC

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 1974	14	7

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	Special Report 74-1
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY

13. ABSTRACT

To confirm or refute the existing regulation requiring a 24 hour interval between diving and flying, dogs were exposed to increased ambient pressures equivalent to water depths encountered in normal professional and recreational diving. The animals were subsequently exposed to reduced pressures comparable to those experienced by naval aircrew members. Various times between hyperbaric and hypobaric episodes were evaluated. The experimental animals were examined using the following methods: (1) Clinical signs; (2) clinico-pathologic determinations; (3) pulmonary interstitial fluid volume; (4) gross pathology; and (5) light microscopy. Evidence of decompression sickness was found. Insofar as the findings may apply to man, a period greater than 12 hours should elapse between diving and flying. An experimental animal species more closely related to man is being examined in larger numbers to precisely define the safe interval.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Unclassified

Security Classification

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Hyperbaric						
Hypobaric						
Decompression sickness						
Canine						
Histopathology						
Pulmonary edema						

DD FORM 1 NOV 68 1473 (BACK)
(PAGE 2)

1a

Security Classification

Approved for public release, distribution unlimited.

HYPERBARIC-HYPOBARIC INTERACTIONS AS THEY RELATE TO COMPRESSED AIR DIVING AND AVIATION: CANINE EXPERIMENT

James L. Kupper, Walter P. Trevethan, and Richard J. Brown

SPECIAL REPORT 74-1

Approved by

Ashton Graybiel, M. D.
Assistant for Scientific Programs

Released by

Captain N. W. Allebach, MC, USN
Officer in Charge

November 1974

Naval Aerospace Medical Research Laboratory
Pensacola, Florida 32512

ib

SUMMARY *

To confirm or refute the existing regulation requiring a 24 hour interval between diving and flying, dogs were exposed to increased ambient pressures equivalent to water depths encountered in normal professional and recreational diving. The animals were subsequently exposed to reduced pressures comparable to those experienced by naval aircrew members. Various times between hyperbaric and hypobaric episodes were evaluated. The experimental animals were examined using the following methods: (1) Clinical signs; (2) clinico-pathologic determinations; (3) pulmonary interstitial fluid volume; (4) gross pathology; and (5) light microscopy. Evidence of decompression sickness was found. Insofar as the findings may apply to man, a period greater than 12 hours should elapse between diving and flying. An experimental animal species more closely related to man is being examined in larger numbers to precisely define the safe interval.

---

* The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

INTRODUCTION

Decompression sickness has been studied extensively in relation to diving, and satisfactory preventive measures have been instituted, i.e., proper decompression periods. Decompression sickness associated with aviation has also been recognized and certain preventive measures applied. While decompression sickness has been studied in its relationship between underwater activities and flying, results have been based on clinical observations of animals (3) and subjective reports from human volunteers (2). Typical signs for dogs included: scratching the skin, limping, lifting of an extremity, respiratory difficulty and change in disposition. Human subjects reported cutaneous itching, joint pain, neurological symptoms, or "chokes" as indicative of decompression sickness. While these are accepted methods they are certainly subject to errors of interpretation and do not detect any asymptomatic cases or so-called "silent bends." The present study was not so limited, but encompassed physiological measurements and pathologic studies of the animal tissue. The approach was to expose animals to increased ambient pressures equivalent to water depths encountered in normal professional and recreational diving. The animals were subsequently exposed to reduced pressures comparable to those experienced by naval aircrew members. Various times between hyperbaric and hypobaric episodes were evaluated to determine the safe interval.

METHOD

Subjects

Sixteen adult male dogs were utilized in the initial experiment. These animals were either from the NAMRL breeding colony or received from another military institution in Washington, D. C. area. They were randomly paired and selected for the various pressure profiles.

Apparatus

A small 180 liter cylindrical chamber was used for overcompression. Compressed air was supplied by a standard Hookah diver's compressor. The air flow through the chamber was maximized by allowing the full output of the compressor to enter the chamber and controlling the pressure by restricting the outflow. At the pressure equivalent of 45 ft. of sea water the flow was 65 liters per minute.

A large hypobaric chamber was used and the animals were accompanied to altitude by two or more investigators. The animals could thus be closely observed during the simulated flight and various sampling and test procedures could be accomplished with relative ease.

Each animal was fitted with an oxygen mask (Figure 1) during the hypobaric exposure and allowed to breathe through a standard aviator's type regulator set in the normal oxygen configuration which provides O_2 at a partial pressure equivalent to ground level. An anaerobic arterial sampling device as described by Smith and Ramsey (7) was used in the measurement of pulmonary interstitial fluid volume.

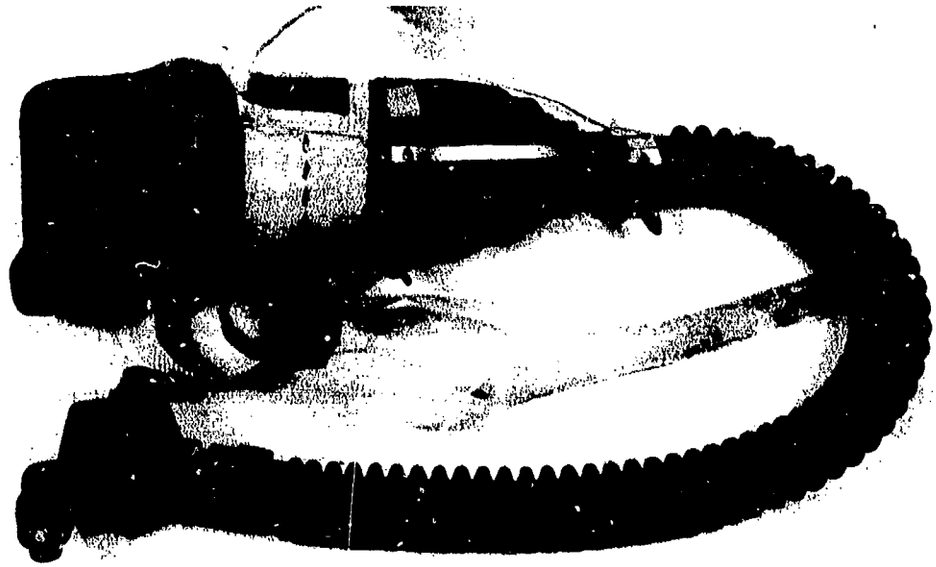


Figure 1

Canine oxygen mask

PROCEDURE

The animals used in this experiment were housed in outdoor kennels. The Pensacola area is endemic for the mosquito borne metazoan canine parasite, Dirofilaria immitis, or heartworm. One month prior to use in the experiment each animal received a therapeutic regimen for heartworms consisting of four intravenous injections of sodium caparsolate over a three day period (4).

The dogs were divided into eight groups (Table I). Group A consisted of sea level controls. Group B control dogs were overcompressed to 20 psig (45 ft. sea water equivalent) for 2 hours. Group C controls consisted of dogs decompressed to 15,000 feet for 3 hours. Group D was overcompressed as was Group C, and then immediately decompressed as was Group B. Groups E through H had intervals between over-compression and decompression of 12, 24, 36, and 48 hours respectively. Every animal in each group was evaluated along the 5 parameters described below:

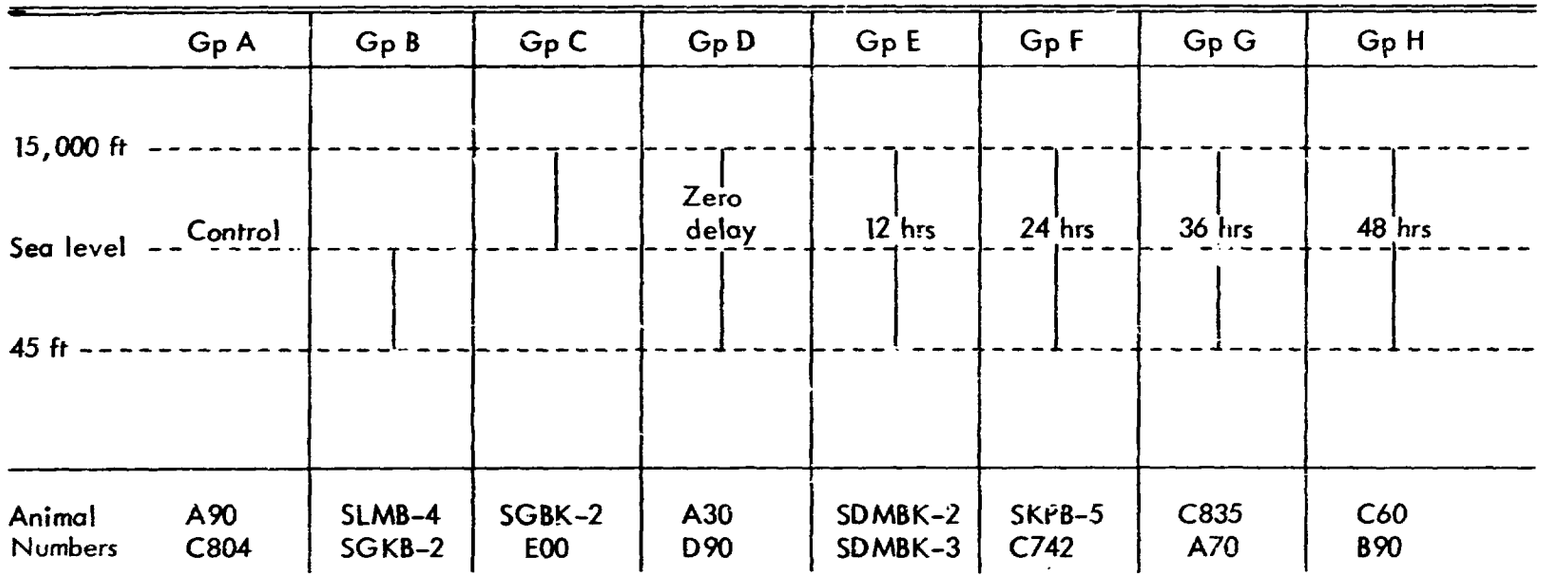
1. Clinical signs. As outlined by Furry (3) the animals were carefully observed for signs of itching, joint pain, respiratory difficulty, behavioral and other central nervous system changes.

2. Clinico-pathologic determinations. The following parameters were measured in all animals: Red blood cell count, packed cell volume, hemoglobin concentration, white blood cell count, differential white blood cell count, blood urea nitrogen, serum glutamic-oxalacetic transaminase concentration, serum glutamic-pyruvic

Table I

Hyperbaric–Hypobaric Interaction

Project Protocol



	Gp A	Gp B	Gp C	Gp D	Gp E	Gp F	Gp G	Gp H
Animal Numbers	A90 C804	SLMB-4 SGKB-2	SGBK-2 E00	A30 D90	SDMBK-2 SDMBK-3	SKPB-5 C742	C835 A70	C60 B90

transaminase concentration, serum lactic dehydrogenase concentration, plasma protein concentration, and serum creatine phosphokinase concentration. Pre- and post-experimental blood sampling was accomplished on each animal.

3. Pulmonary interstitial fluid volume. This volume was measured by the method of Smith and Ramsey (7) in which the transit times of two radioisotopes through the lungs are compared as they relate to the volume of the compartments traversed. Albumin I^{131} and tritiated water were used. The albumin remains within the vascular system while the tritiated water exchanges freely with the interstitial fluids. In order to inject and recover the isotopes, one indwelling silicone rubber catheter was inserted via the left jugular vein into the right atrium and one similar catheter was inserted via the left carotid artery into the proximal aorta. Catheter positions were verified both by contrast radiography and pressure profiles. These catheters were placed three days prior to the actual experiment to allow recovery from the effects of surgery and anesthesia. An injection and collection of isotopes was done at ground level immediately before the hyperbaric, hypobaric, or sham exposure. A second determination was made in the hypobaric chamber following three hours at 15,000 feet equivalent pressure or ground level in the case of controls.

4. Gross pathology. At the termination of the hypobaric exposure each animal was humanely killed with a rapidly administered intravenous mixture of barbiturates at the recommended dosage. A complete necropsy was performed at altitude and special attention was given to examination of the vascular system for gaseous emboli and catheter tip positions. Control animals were necropsied following hyperbaric, hypobaric, or sham exposure.

5. Light microscopy. Tissues were collected at simulated altitude and allowed to remain under hypobaric conditions in 10% buffered formalin for several hours following the necropsy in order to fix lesions which would be subject to change on recompression. Tissues collected from sea level and diving controls were fixed in 10% buffered formalin at sea level. Fixed tissues were embedded in paraffin blocks, sectioned on a rotary microtome at 6-7 microns, placed on glass slides and stained with hematoxylin and eosin.

RESULTS

Results are as follows:

1. Clinical signs. Significant clinical signs were seen in the zero delay and twelve hour delay animals. One zero delay and one 12 hour delay dog showed rapid labored breathing only. The other zero delay dog showed labored breathing, elbow abduction, and reluctance to bear weight on one hind limb. The remaining 12 hour delay dog exhibited rapid labored breathing, followed by vigorous fighting of the mask, rapid rolling along the longitudinal axis, penile erection, palpable subcutaneous crepitation of the lateral thorax, and cyanosis, even while receiving one hundred percent oxygen. Death occurred in this latter case. The other six groups of animals showed no clinical signs.

2. Clinico-pathologic determinations. A statistical comparison was made between the pre- and post-experimental values within each group. No significant differences were observed.

3. Pulmonary edema. An increase in pulmonary interstitial fluid volume is thought to be an early change in pulmonary edema. Measurements for this volume were made on fifteen (one death) of the sixteen dogs. Statistical analysis revealed no significant increases in pulmonary interstitial volume in any of the groups. Histologically, pulmonary edema was not a consistent finding in our studies of decompression sickness in the dog. An interesting sidelight to the pulmonary interstitial volume measurement study was an indwelling catheter tip myocarditis. Three to five days of catheter tip irritation to the atrium caused focal myocardial hemorrhage and fibrosis in several dogs.

4. Gross pathologic findings. The animal which died revealed diffuse severe gaseous emboli engorging the inferior vena cava, diffuse sub-pleural and sub-epicardial petechiae and ecchymoses, and a pink froth throughout the respiratory tree. The other animals were free of gross lesions associated with decompression sickness.

5. Light microscopy. Changes seen in animals with acute decompression sickness were generally confined to the medium sized arteries in the lung, liver and kidney. The change was most prominent in the lung (Figures 2 and 3). The basic change was a dissection and dissociation of the smooth muscle, reticular, collagenic, and delicate elastic fibers of the outer tunica media and the tunica adventitia. There was no particular distribution pattern to this arterial change in the lungs or liver. In the kidney the affected arteries were usually near the cortico-medullary junction.

Since the number of animals in each experimental canine group of the pilot study was small (two), and the pressure profile was not severe, interpretation of the lesions was difficult. In order to clarify and facilitate identification of changes, three groups of five rats each were examined. Fifteen adult, slightly obese Sprague-Dawley rats were utilized. Ten rats were overcompressed for a period of 2 hours at 35.6 psig (80 feet sea water) in an overcompression chamber. The animals were returned to sea level without decompression stops. This is a violation of the human decompression tables. Immediately upon reaching sea level, five of these animals were humanely killed. The remaining five were taken to an altitude of 15,000 feet. After a period of 2 hours at this altitude, three rats had died with signs of decompression sickness. The remaining two, both showing posterior ataxia, were humanely killed with an intravenous injection of barbiturates. Tissues from all ten were harvested for light microscopic examination. Tissues from a control group of five animals, exposed to neither hyperbaric nor hypobaric environment, were also submitted to the pathology laboratory. Pulmonary vascular lesions similar to those illustrated in Figures 2 and 3 were found in the experimental rats. The occurrence of these lesions is presented in Table II. As expected, the group taken directly from the hyperbaric to the hypobaric environment exhibited the most marked changes. The severe changes seen in the zero delay rats did not occur consistently in any of the eight groups of dogs.

Figure 2

Photomicrograph of Canine Pulmonary Artery Showing Separation of Elastic Fibers of the Tunica Adventitia and Tunica Media. H + E X 40.

Figure 3

Higher Power of Figure 2. H + E X 200.

Table II

Pulmonary Vascular Lesions in Rats

Group	Animals with Lesions	Number in Group
Zero Delay	5	5
Diving Control	2	5
Sea Level Control	0	5

It has been suggested that the histopathological lesions observed could possibly be artifactual, produced by opening the carcass for necropsy at altitude. An alternate method of preserving and recovering tissue is to wash the intact animal free of blood by flushing saline through the vascular system and then filling the system with formalin. The tissues are thus perfused and fixed before the carcass is opened. The same vascular lesions were found in two animals overcompressed, decompressed and prepared in this way.

DISCUSSION

This study was prompted by a strong conviction among the authors that signs or symptoms of decompression sickness in animals are not reliable endpoints for evaluating pressure profiles. Dogs were chosen for the pilot study for several reasons: 1) The previous work on the safe interval between diving and flying had been done with dogs, 2) a special oxygen mask for canines had already been developed, and 3) a colony of standardized and well defined canines was available. The human dive profile which was selected was considered to be conservative for dogs, as the canine has been shown to be more resistant to decompression sickness than is man (3, 6). Furry (3) conducted bends threshold dives in order to determine profiles for individual dogs which would produce signs of decompression sickness. By easing the profile somewhat, he could then produce a "near bends" dive. However, it is generally felt that an individual threshold can change, and the possibility of producing symptoms or changes from the dive alone would then exist.

In retrospect, the choice of the canine as an experimental model for decompression sickness was unfortunate to a certain extent, since the dog's respiratory system serves a dual purpose. Not only does the respiratory system serve the conventional role of gas exchange, but it also is involved in thermo regulation (panting). Temperature changes within the decompression chamber caused excessive variations in breathing patterns and activity, and the oxygen mask placed over the dogs' muzzle did not allow panting and thus normal heat loss from the animal's body. Another problem, not peculiar to the dog,

arises when any animal is used in the measurement of pulmonary interstitial fluid volume. Ramsey (5) has demonstrated a wide variation in volume between measurements made at different phases of the respiratory cycle. Since it was impossible to induce breath holding in the dog during these measurements, accurate interstitial fluid volumes were not achieved.

The interpretation of the vascular lesions was difficult since each group consisted of only two animals. The groups were not larger since this experiment was considered to be a pilot study. Chryssanthou (1) reported similar lesions following decompression sickness in mice. While we believe the dissection and dissociation of the vascular wall is a real lesion, and certainly is present in decompression sickness, it is not pathognomonic. Similar but less extensive lesions have been seen by the authors in animals considered to have been stressed, but never to the degree or extent seen in severe cases of decompression sickness. Further elucidation of the significance of this lesion in decompression sickness is presently being sought in experiments with non-human primates.

CONCLUSION

Dogs exposed to a conservative hyperbaric profile, followed immediately or within 12 hours by exposure to a simulated 15,000 feet altitude, showed symptoms, gross lesions and histopathologic changes associated with decompression sickness. Animals delayed for longer than 12 hours showed no changes except for possible histopathologic perivascular lesions that are difficult to interpret in such a small experimental group. Measurement of pulmonary extravascular water volume was an unsatisfactory procedure for the canine under the conditions of this experiment.

Results indicate that some period greater than 12 hours should elapse between hyperbaric and hypobaric exposure to prevent decompression sickness. In order to more precisely identify the minimal safe interval, a larger group of experimental animals, phylogenetically closer to man, is being examined.

REFERENCES

1. Chryssanthou, C., Kalberer, J., Jr., Kooperstein, S., and Antopol, W., Studies on dysbarism II. Influence of bradykinin and "bradykinin-antagonists" on decompression sickness in mice. Aerospace Med., 35:741-746, 1964.

2. Edel, P. O., Carroll, J. J., Honaker, R. W., and Beckman, E. L., Interval at sea-level pressure required to prevent decompression sickness in humans who fly in commercial aircraft after diving. Aerospace Med., 40:1105-1110, 1969.

3. Furry, E., Reeves, E., and Beckman, E., Relationship of scuba diving to the development of aviators' decompression sickness. Aerospace Med., 38: 825-828, 1967.

4. Jackson, R. F., Two day treatment with thiacetarsamide for canine heartworm disease. J. Am. Vet. Med. Assn., 142:23-26, 1963.

5. Ramsey, L. H., Puckett, W., Jose, A., and Lacy, W. W., Pericapillary gas and water distribution volumes of the lung calculated from multiple indicator dilution curves. Circulation Res., 15:275-287, 1964.

6. Reeves, E., and Beckman, E. L., The incidence of decompression sickness in dogs following 7, 12, 18, and 24-hour saturation dives with no-stop decompression. Research Report No. 4, Research Task No. MF 011.99-1001, April 1966, Bethesda, Md.

7. Smith, R. F., and Ramsey, L. H., The measurement of pulmonary extravascular water volume during exposure to simulated high altitude. NAMRL-1176. Pensacola, Fla.: Naval Aerospace Medical Research Laboratory, 1973.